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Syntheses and Biological Activities of Uridine Nucleoside Derivatives

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Abstract

Many nucleoside compounds such as 5-halogen substituted uridine, 5'-amino-5'-deoxyuridine conjugates of amino acid, peptide, and penicillin G, 5'-monophosphate uridine derivatives and 5'-monophosphate uridine-fatty acid derivatives were chemically synthesized and their antifungal, antibacterial, and antitumor activities were tested. 5-Bromo-2',3'-O-isopropylideneuridine(6) inhibited the growth of *Trichophyton rubrum* at 0.2 μg/ml of MIC. 5'-Amino-5'-deoxyuridine-penicillin G(19), 5'-amino-5'-deoxyuridine-cyclo(Phe-Asp)(20), and 5-iodo-5'-amino-5'-deoxyuridine-penicillin G(22) had antibacterial activity(MIC was 6.25 μg/ml against *S. aureus*) and the latter two nucleoside compounds were the most antitumor derivatives(their IC_{50} against *L5178Y murine lymphoma cell* was 6.5 μg/ml).

Key words : uridine nucleoside derivative, biological activity

Introduction

Study on the chemotherapeutic agents from salvarsam to AZT had been developed rapidly. They directly suppressed the *in vivo* growth of pathogenic bacteria and cured many diseases to prolong the life of human being [7]. In this study the 5'-hydroxyl group of uridine derivative designed as anti-viral agent [1, 9] was changed to amino group. A little antibacterial cyclicpeptide [6] was coupled to recently deactivated penicillin G[4] by chemical method [2] in order to increase the biological activity. Nucleoside chemotherapeutic agents used today had many problems, among which involved the revelation of tolerance and toxicity for host cell. Especially the modified nucleoside derivatives were not proper substrate for kinase and then the substrate-specific activity for kinase was decreased, so that the triphosphate-type nucleoside derivatives required in the biosynthesis of DNA and RNA were not formed. This problem could be solved by the phosphorylation and the coupling of transport molecule to nucleoside derivative [11]. Transport molecule made the active transport easy [12, 14], mitigated the toxicity of drug [4], or acted especially in the cell membrane of pathogenic microorganism to show good *in vivo* biological activity [8, 10].

The 5-position of uridine was substituted by halogen and Z-amino acid, peptide, and penicillin G as transport molecule was coupled to 5'-amino-5'-deoxy-2',3'-O-isopropylidene uridine and 5'-monophosphate derivatives of uridine were synthesized in this study. The coupled or synthesized nucleoside compounds were *in vitro* assayed for antifungal, antibacterial, and antitumor activities in terms of MIC(μg/ml) and IC_{50}(μg/ml).

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Materials and Method

Reagents and Instruments

Uridine, 2,2-dimethoxypropane, bis-(p-nitrophenyl) phosphate hydrate, p-toluenesulfonyl chloride, EDC, and HOBt were purchased from Aldrich Co. N-Halosuccinimide, triethylphosphate, and silicagel were obtained from Fluka Co. Penicillin G sodium salt, DCC, and DEAE cellulose were obtained from Sigma Chemical Co. Reduced glutathione, Kiesegel 60 F254, and 60 PF254 were purchased from Merck. L-amino acid, phosphoryl chloride, palmitic acid, and stearic acid were purchased from Hayashi Co.

The identification of reaction product was performed with UV lamp(254 nm) and m.p. was measured with electrothermal capillary melting point apparatus. IR spectra were obtained on Hitachi 260-30 spectrometer using KBr pellet. ^{1}H-NMR spectra were measured with Varian EM-30A NMR spectrometer using TMS as internal reference. ^{31}P-NMR was done with the aid of POSCO and Mass spectra, LG Institute. The MIC was determined by using Spectronic 20(Bausch and Lomb Inc., Rochester N.Y.).

Antifungal and antibacterial activity assay

The minimal inhibitory concentrations(MICs) for the growth of the microorganisms by the synthesized nucleoside compounds were measured using the broth dilution method [13, 15] for *Trichophyton rubrum, Escherichia coli,* and *Staphylococcus aureus.*

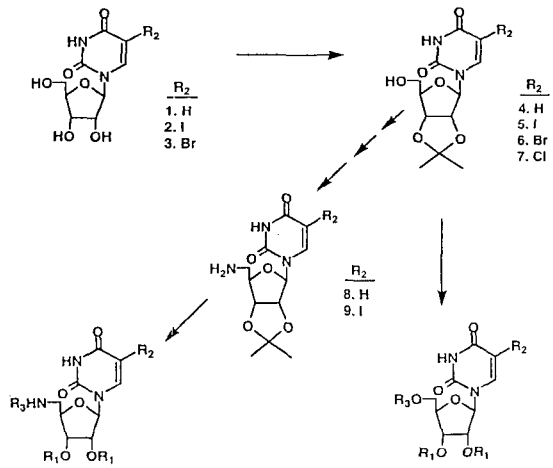
Antitumor activity assay

The IC_{50}s of the synthesized nucleoside compounds were measured for *Mouse lymphoblastoma L5178Y cell.*

Syntheses of nucleoside derivatives

Nucleoside derivatives were synthesized as the following Scheme I and II.

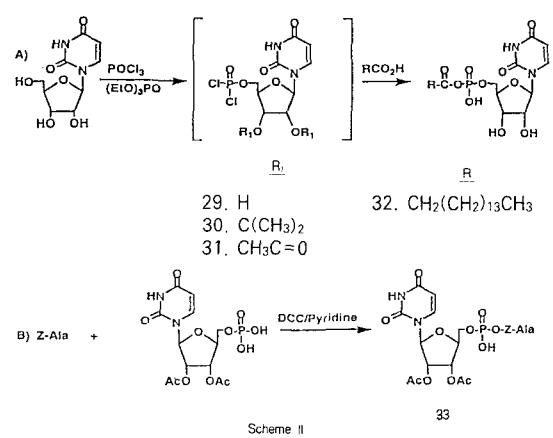
Synthesis of 5-Bromouridine(3). Ten ml of DMF was



R1	R2	R3
10. $C(CH_3)_2$,	H,	Z-Phe
11. $C(CH_3)_2$,	H,	Z-Asn
12. $C(CH_3)_2$,	H,	Z-Ser
13. $C(CH_3)_2$,	H,	Z-Lys(Z)
14. $C(CH_3)_2$,	H,	Z-Phe-Leu
15. $C(CH_3)_2$,	H,	Z-Glu-Tyr
16. $C(CH_3)_2$,	H,	C(Phe-Asp)
17. $C(CH_3)_2$,	H,	C(Phe-Glu)
18. $C(CH_3)_2$,	H,	Ala
19. H,	H,	Penicillin G
20. H,	H,	C(Phe-Asp)
21. $C(CH_3)_2$,	I,	Penicillin G
22. H,	I,	Penicillin G

R1	R2	R3
23. $C(CH_3)_2$,	H,	Z-Phe
24. $C(CH_3)_2$,	H,	Z-Asn
25. $C(CH_3)_2$,	H,	Ser
26. $C(CH_3)_2$,	H,	Lys
27. $C(CH_3)_2$,	H,	Penicillin G
28. H,	I,	Penicillin G

Scheme I. Synthetic Procedure of Uridine nucleoside derivatie(1)



Scheme II. Synthetic Procedure of Uridine nucleoside derivatie(2)

added to uridine(4.10 mmol) and NBS(4.92 mmol) and reacted at room temperature until the solution was changed yellow. The solvents were concentrated and crystallized with $CHCl_3$. The white solid was washed with EtOH and recrystallized to obtain white crystal. Yield 83%: mp 191-193 °C.

Synthesis of 5-bromo-2',3'-O-isopropylidene uridine (6). 6 was obtained from the 2',3'-O-isopropylidene uridine(4) starting material [3] by using the synthetic method of 5-bromouridine(3). It was crystallized with H_2O and recrystallized with diethyl ether. Yield 68% : mp 225-228 °C.

Synthesis of 2',3'-O-isopropylidene-5'-O-toluenesulfonyl uridine. 2',3'-O-isopropylidene uridine(4) and p-tolunesulfonyl chloride(5.2 mmol) were added to the anhydrous pyridine(10 ml) at 0 °C by the method of Tipson. After the addition of MeOH, the residual pyridine was coevaporated with toluene. Ice-H_2O was added, extracted with $CHCl_3$, dried with Na_2SO_4, concentrated, and then chromatographed with silicagel column using $CHCl_3$-Me_2CO(7:3) elution solvent. The eluted material was recrystallized with diethyl ether. Yield 78% : mp 89-90 °C ; IR(KBr) 2750 (5'-O-Ts), 1650 (amide C=O), 1490 and 1370 cm^{-1}($(CH_3)_2C$-) ; ^{1}H-NMR ($CDCl_3$) δ 8.67(d, 1H, C_6H), 7.74 and 7.35(s, 4H, aromatic protons), 4.83(m, 2H, 5'-CH_2O-Ts), 2.30(s, 3H, CH_3 of p-Ts), 1.35 and 1.55(s, 3H, $(CH_3)_2C$-), Mass : m/e 438 ($C_9H_{22}N_2O_8S$).

Synthesis of 5'-azido-5'-dioxy-2',3'-O-isopropylidene uridine. 2',3'-O-isopropylidene uridine-5'-O-p-toluenesulfonyl chloride(2.2 mmol) and NaN_3(4.4 mmol) were dissolved in anhydrous DMF(10 ml) and heated at 75-80 °C. DMF was removed at reduced pressure and coevaporated with EtOH. The residue was triturated with H_2O and acidified with glacial acetic acid, and then extracted with $CHCl_3$. The $CHCl_3$ layer was washed with 5% $NaHCO_3$. The organic layer was dried with Na_2SO_4 and concentrated at reduced pressure, and then crystallized with diethyl ether. Yield 78% : mp 161-163 °C ; IR(KBr) 2101(azide), 1670(amide C=O), 1490 and 1370 cm^{-1}($(CH_3)_2C$-) ; ^{1}H-NMR(DMSO-d_6) δ 8.65(d, 1H, C_6H), 5.80(d, 1H, $C_1'H$), 4.41-4.76(m, 3H, $C_2'H$, $C_3'H$, $C_4'H$), 3.54(m, 2H, 5'-CH_2N_3), 1.27 and 1.47(s, 3H, $(CH_3)_2C$-), Mass : m/e 308 ($C_{12}H_{14}N_5O_8$).

Synthesis of 5'-amino-5'-deoxy-2',3'-O-isopropylidene uridine(8). 5'-azido-5'-O-deoxy-2',3'-O-isopropylidene uridine (2.6 mmol) was dissolved in EtOH(50 ml) and added Pd black to hydrogenate(3.5 kg/cm^2 pressure) by Tomson's method. The product was crystallized with diethyl ether and the white crystal was obtained. Yield 81% : mp 221-225°C ; IR(KBr) 3100-3500(medium, primary NH), 1680(C=O), 1500 and 1430 cm^{-1}($(CH_3)_2C$-) ; ^{1}H-NMR(DMSO-d_6) δ 7.51(d, 1H, C_6H), 4.41-4.76(m, $C_2'H$, $C_3'H$, $C_4'H$), 3.21 and 3.70(m, 2H, 5'-CH_2N_3), 1.32 and 1.55(s, 3H, $(CH_3)_2C$-).

Synthesis of 5'-amino-5'-deoxy-2',3'-O-isopropylidene uridine-5'-Z-Lys(13). Z-Lys(1.7 mmol), HOBt(3.5 mmol), and DCC(3.5 mmol) were dissolved in anhydrous EtOAc (50 ml) at 0°C by Schinazi's method[16]. 5'-amino-5'-deoxy-2',3'-O-isopropylidene uridine(1.3 mmol) in CH_2Cl_2 was added and stirred. The solution was filtered, removed the excess DCU, and washed with $NaHCO_3$ and 10% citric acid to obtain white solid. The solid was recrystallized to obtain 13. Yield 82% : IR(KBr) 3350 (medium, -NH), 1680(C=O), 1460 and 1370 cm^{-1}($(CH_3)_2C$-) ; ^{1}H-NMR(DMSO-d_6) δ 7.70(d, 1H, C_6H), 7.25(s, 5H, phenyl), 5.05(s, 2H, benzyl), 4.15(m, 2H, 5'-CH_2), 1.25 and 1.50(ss, 6H, $(CH_3)_2C$-).

Synthesis of 5'-amino-5'-deoxy uridine-cyclo(Phe-Asp) (20). 5'-amino-5'-deoxy uridine (3.16 mmol), cyclo(Phe-Asp)(9.48 mmol), N-hydroxysuccinimide(9.48 mmol), and DCC(9.48 mmol) were dissolved in anhydrous pyridine (40 ml) and obtained the product. Yield 76% : IR(KBr) 3500(medium, -NH), 3100(aromatic C-H), 2990(alkane C-H), 1660(amide C=O), 1600 and 1490 cm^{-1}(aromatic C=C) ; MS m/e 487($C_{22}H_{25}N_5O_8$).

Synthesis of 5-iodo-5'-amino-5'-deoxy uridine-penicillin G(22). 5-Iodo-5'-amino-5'-deoxy uridine(9, 3.16 mmol), penicillin G sodium salt(9.48 mmol), and DCC(9.48

mmol) were dissolved in anhydrous pyridine(40 ml) and obtained 22. 90% CF_3COOH was added to 21 and reacted at room temperature, concentrated at reduced pressure, and then obtained the product. Yield 85% : IR(KBr) 3100(medium, -NH), 3050(aromatic C-H), 2900 (alkane C-H), 1640-1700(amide C=O), 1600 and 1470 cm^{-1}(aromatic C=C) ; MS m/e 684($C_{25}H_{28}N_5O_8Sl$).

Synthesis of 5',2',3'-O-isopropylidene uridine-penicillin G(27). 2',3'-O-isopropylidene uridine(4, 7.5 mmol), penicillin G sodium salt(11.2 mmol), N-hydroxysuccinimide (11.2 mmol), and DCC(11.2 mmol) were dissolved in anhydrous pyridine(40 ml) and reacted for 24h at ice-bath. The precipitate was filtered and the solvents were concentrated, and crystallized with H_2O. The crystallized precipitate was filtered and concentrated to separate with PLC. The product was recrystallized with $CHCl_3$. Yield 75% : IR(KBr) 3100-3500(medium, -NH), 3050(aromatic C-H), 2860(alkane C-H), 1680(amide C=O), 1600-1470(aromatic C=C), 1490 and 1370 cm^{-1}($(CH_3)_2C$-).

Synthesis of 5-iodouridine-penicillin G(28). 5-Iodo-2',3'-O-isopropylidene uridine(7.5 mmol), penicillin G sodium salt(11.2 mmol), N-hydroxysuccinimide(11.2 mmol), and DCC (11.2 mmol) were dissolved in anhydrous pyridine(40 ml) and obtained 28 as the same method of 27. 90% CF_3COOH was added and reacted at room temperature. The product was concentrated and obtained the product. Yield 74% : IR(KBr) 3100-3500 (medium, -NH), 3050(aromatic C-H), 2860(alkane C-H), 1660(amide C=O), 1600 and 1470 cm^{-1}(aromatic C=C) ; MS m/e 694($C_{25}H_{28}N_4O_9Sl$).

Synthesis of uridine 5'-monophosphate(disodium salt, 29). $POCl_3$(7.42 mmol) was added to redistilled $(EtO)_3PO$ at 10 - 0 °C. Uridine(4.0 mmol) in $(EtO)_3PO$ was added and reacted for 3h at 0 - 5 °C. Ice-H_2O(20 ml) was added dropwise and neutralized with conc. NH_4OH to pH 7.0. The mixture was washed with Et_2O-petroleum ether(1:1) and dried under reduced pressure to obtain white solid. The solid was triturated to form sodium salt type. Analytical sample was obtained after DEAE cellulose column chromatography. Yield 75% : IR(KBr) 3100(NH_2), 1610(C=O), 1250(P=O), 1080 and 1055 cm^{-1} (POC) ; ^{31}P-NMR δ 0.98(s, 1P).

Synthesis of 2',3'-O-diacetyluridine 5'-monophosphate (disodium salt, 31). Ac_2O 30 ml and anhydrous pyridine 60 ml were added to 29(5 mmol) and reacted for 18h at room temperature. After the identification of the reaction using TLC, ice-H_2O 20 ml was added in ice-bath. The mixture was stirred for 3h at room temperature and dried at reduced pressure to obtain 31. The product was coevaporated with anhydrous pyridine and removed H_2O. Yield 56% : IR(KBr) 3400(NH), 1720(C=O), 1657 and 1610(C=O, C=C), 1220(P=O), 1080 and 1040 cm^{-1}(POC) ; ^{1}H-NMR(DMSO-d_6) δ 9.48(d, 1H, C_6H), 5.1-5.6(broad), 3.0(s, 2H, $C_5'CH_2$), 1.9-2.2(t, 6H, -$(CH_3)_2$).

Synthesis of 2',3'-O-diaectyluridine 5'-monophosphate Z-Ala(disodium salt, 33). 31(2.5 mmol), Z-Ala(5.0 mmol), and DCC(10 mmol) were dissolved in anhydrous pyridine 20 ml. The solution was concentrated at reduced pressure and anydrous pyridine(25 ml) was added. This step was performed again and again and reacted for 5 days. The excess DCU was filtered and washed with pyridine. The filtrate was concentrated, separated with PLC, and obtained the sodium salt type. Yield 65% : IR(KBr) 3400(NH_2), 1740(C=O), 1655 and 1610(C=O, C=C), 1230(P=O), 1080 and 1050 cm^{-1}(POC) ; ^{1}H-NMR (DMSO-d_6) δ 7.42(s, 6H, benzyl), 6.8(d, 1H, C_6H), 5.0(s, 2H), 3.1(s, 2H, $C_5'CH_2$), 1.8-2.1(t, 6H, -$(CH_3)_2$), 1.1-1.2(d, 3H, CH_3).

Results and Discussion

The antifungal and antibacterial activities of the synthesized nucleoside compounds were measured using the broth dilution method for the growth of *Trichophyton rubrum, Escherichia coli,* and *Staphylococcus aureus,* and then the antitumor activities of the synthesized nucleoside derivatives were measured for *Mouse lymphoblastoma L5178Y cell.* The results were presented in Table

1, 2, and 3.

The most antibacterial and antitumor compounds were 20 and 22(MIC and IC_{50} were 6.25 μg/ml and 6.5 μg/ml, respectively, Table 2 and 3). The MIC of compound 6 was the lowest(0.2 μg/ml) for *Trichophyton rubrum*(Table 1). The results of the antibacterial activity test showed that the synthesized nucleoside-peptide(20) and the nucleoside-penicillin G derivatives(19, 22) had similar activity as that of penicillin G(Table 2). These results suggested that the inhibition of bacterial growth was proceeded as the Prusoff's mechanism. The nucleoside-peptide and nucleoside-penicillin G derivatives were separated as peptide, penicillin G, and nucleoside derivative and then coupled to RNA chain abnormally and induced the breaking of it in the biosynthesis of RNA, or inhibited the transcription because of the impossible hydrogen-bonding between bases. In the antibacterial activity test for simple nucleoside derivatives and their phosphorylated compounds, the nucleoside derivatives showed better activities(Table 2). Most nucleic acid and its derivatives were phosphorylated as 5'-monophosphate by viral and cellular kinase in cell and changed to 5'-triphosphate in the final step so that it was biologically active. These phosphorylated nucleoside derivatives were changed more easily to di- or tri-phosphate than nonphosphorylated derivatives so that they induced the chain termination after they combined to RNA or they suppressed the growth of bacteria after they directly inhibited the activity of RNA polymerase.

Table 1. Antifungal activities of synthesized compounds for *Trichophyton rubrum*

Compound No.	MIC (μg/ml)
2	1.56
3	1.56
6	0.2
5-Fluorouracil	0.05
Amphotericine B	7.3
Nystation	31.2

Table 2. Antibacterial activities of synthesized compounds for *E coili* and *S. aureus*

Compound No.	MIC (μg/ml)	
	E. coli	*S. aureus*
6, 30, 31	> 100	50
8, 23, 24, 25, 26	> 100	100
10, 11, 12, 13	200	100
15	200	100
16	200	50
17	> 100	25
18, 32, 33	> 100	100
19, 20, 22	> 100	6.25
27, 28	> 100	12.5
29, 32	200	200
Penicillin G	> 100	6.25
Polyoxine	200	200

Table 3. Antitumor activities of synthesized compounds for *L5178Y murine lymphoma cells.*

Compound No.	IC_{50} (μg/ml)
2, 3, 4, 5, 8	> 30.0
6	27.0
7	25.0
19	18.0
20, 22	6.5
21	13.5
27	9.0
5-iodouridine	> 30.0

The new potent biologically active agents ; 5-halogen substituted uridine compound, 5'-aminouridine derivatives, and peptide-penicillin G coupled nucleoside agents might have the synergistic effect, so the coupled or synthesized uridine nucleoside derivatives in this study might be medically active agents which could overcome the defects of pre-developed chemotherapeutic agents.

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초록 : Uridine Nucleoside 유도체의 합성과 생물 활성

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5-halogen substituted uridine, amino acid, peptide 및 penicillin G의 5'-amino-5'-deoxyuridine conjugates, 5'-monophosphate uridine 유도체, 5'-monophosphate uridine-fatty acid 유도체와 같은 nucleoside 화합물들을 화학적으로 합성한 후 이들의 항진균, 항균 및 항암 활성을 측정하였다. 5-Bromo-2',3'-O-isopropylideneuridine(6)은 *Trichophyton rubrum*의 성장을 억제하였다(MIC : 0.2μg/ml). 5'-Amino-5'-deoxyuridine-penicillin G(19), 5'-amino-5'-deoxyuridine-cyclo(Phe-Asp)(20), 5-iodo-5'-amino-5'-deoxyuridine-penicillin G(22)는 항균적이었고(*S. aureus*에 대해 MIC가 6.25μg/ml) 뒤의 두 nucleoside 화합물은 항암 작용이 가장 강한 유도체이었다(*L5178Y murine lymphoma cell*에 대한 IC_{50}이 6.5μg/ml).

주제어 : uridine nucleoside 유도체, 생물 활성